Use of porous anodic alumina membranes as a nanometre-diameter column for high performance liquid chromatography

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The possibility of using porous anodic alumina membranes as a column for normal-phase high performance liquid chromatography was evaluated using phenol and toluene with mobile phases having different solvent compositions.

Recently, nanostructured materials have attracted much interest in various areas ranging from industry to medical science, and many preparation methods and applications have been reported.[1–6] In particular, porous anodic alumina (PAA) membranes[7–12] have been intensively studied and widely applied as ultrafiltration membranes and templates for nanostructure synthesis because of their ordered structure, superior resistance to organic solvents, and potentially low cost.

PAA membranes consist of a self-assembled honeycomb array of uniformly sized parallel channels. Generally, the diameter of the pores is tunable in the range of about ten to several hundred nanometres, and membrane thickness can range from 10 to 100 μm.

When solutes permeate through the PAA membrane, the interior of the columnar channels interacts with the solute molecules. The transport velocity of solute molecules in the channels is thought to depend on the interaction with the interior. Therefore, the nanochannels in the PAA can be expected to function as chromatographic columns.

Alumina is a popular packing material in high performance liquid chromatography (HPLC), and its chromatographic properties have been widely studied.[13–17] Commonly, chromatographic packing materials are prepared as particles. In order to use the particles as the solid phase in HPLC, it is necessary to pack the particles into the column blank. Thus, ordinary column for HPLC is about 10 to 30 cm long, and it is very expensive. On the other hand, PAA membranes are very thin and very cheap, which is favorable for reducing the size and production cost of analysis systems.

Several studies using PAA membranes as separation matrix have been reported. Sano *et al.* achieved the size separation of DNA on a biochip in electrophoresis.[7] Martin and co-workers used functionalized PAA membranes to separate enantiomers and ionic molecules in a permeation cell.[18,19] Although fine investigations have been conducted in this way, no study using PAA membranes as an HPLC column for separation of solutes has been reported yet.

Here we report that a porous anodic alumina membrane can be used as a nanometre-diameter column in an HPLC system. PAA membranes (Anotop, 100 nm diameter pores, 60 μm thick, Whatman) were obtained commercially. The membrane cartridges were set in an HPLC system as chromatographic columns (Fig. 1), and the retention times of toluene and phenol were measured in a heptane–EtOH mobile phase with changing solvent compositions. The dependence on the number of membranes was also estimated by connecting membrane cartridges in series. The pore diameter of the membranes is very small compared to the membrane thickness (length-to-diameter aspect ratio $\approx$ 600) makes the channels well suited for a chromatographic column.

Previously, we used porous anodic alumina membranes with perpendicularly oriented silica–surfactant nanochannel assembly membranes (NAM) as a chromatographic matrix.[20] In order to pass eluents through the NAM, it is necessary to apply high pressure by means of the pump because the pore diameter of the NAM is very small (3.4 nm). This property is unfavorable for

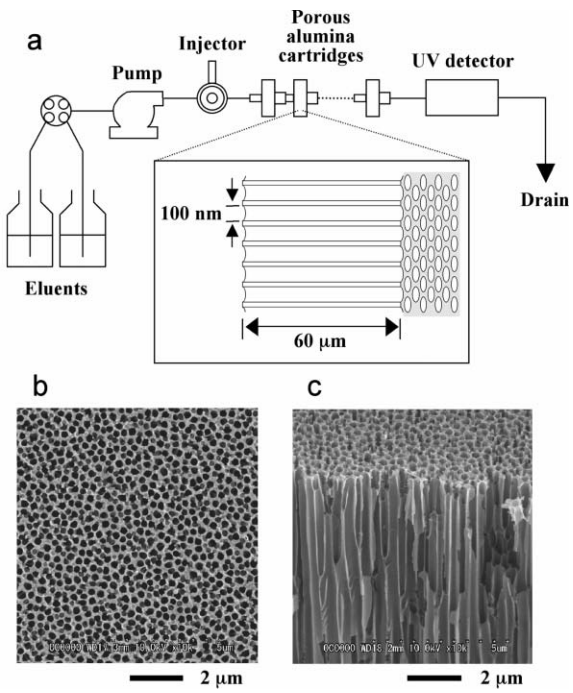

Fig. 1 (a) Schematic illustration of HPLC system. (b) Top view SEM image of PAA membrane. (c) Cross-section SEM image of PAA membrane.

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constructing and operating analysis systems. On the other hand, in the case of PAA membranes, eluents can pass though the membrane easily.

The retention time of phenol is longer than that of toluene (Fig. 2a) indicating that the nanochannels of the PAA membrane can function as nanometre-order chromatographic columns for HPLC. The retention time reflects the strength of the interaction between the solute molecules and the interior of the nanochannels. The polarization of electrons of the p or π orbitals in a strongly positive surface field is reported to play an important role in the interaction between the aromatic solutes and the alumina surface.[21,22] In the case of phenol, the electrons of the hydroxyl group are polarized by the alumina surface field resulting in the formation of an inductive dipole.[24] The inductive dipole interacts with the alumina surface. As a result, phenol molecules interact with nanochannels more strongly than toluene.

The number of membranes also has a large influence on the retention time of solutes. The difference in retention time between toluene and phenol increases with the number of membranes (Fig. 2b). In this experiment, increasing the number of membranes corresponds to lengthening the column in chromatography. In general, the resolving power of a column increases with increasing length of the column. As the column length becomes longer, collisions between the solute molecules and the interior increase, and the solutes are retained longer.

The solvent composition of the mobile phase affects the retention of solutes significantly. Retention times of phenol vary sharply in the low EtOH mole fraction region (<0.04), whereas toluene is insensitive to the EtOH content (Fig. 3). When the mole fraction of EtOH is in the range 0.04–0.1, the retention times of the two solutes are almost equal. The hydroxyl group in the phenol molecule can form hydrogen bonds with EtOH molecules, which reduces the interaction between the alumina surface and phenol. To examine the hydrogen-bonding of solutes in bulk solution, the dependence of the absorption maximum on the solvent composition was measured (Fig. 4). The absorption maximum of phenol shifts to low energy with increasing EtOH mole fraction, which is attributed to the formation of hydrogen bonds.[23]

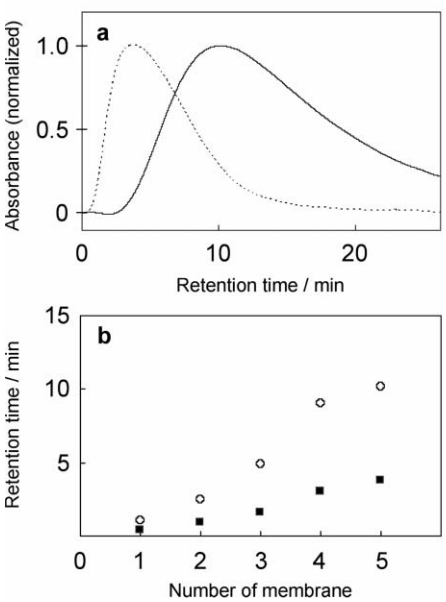

Fig. 2 (a) Chromatogram of toluene and phenol. Dotted and solid lines show toluene and phenol, respectively. Five PAA membrane cartridges were connected in series. The mobile phase conditions were heptane : EtOH = 99 : 1 at a flow rate of 0.6 ml min^{-1}. The injection volume was 50 μl, and concentration of each solute was 10 mM. Detection wavelength was 260 nm. (b) Plot of retention time *vs.* number of membranes: toluene (■) and phenol (○). The mobile phase conditions were the same as in Fig. 2a.

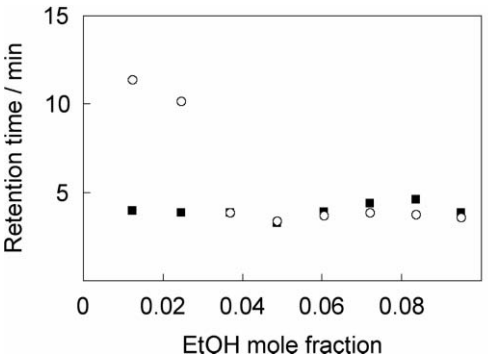

Fig. 3 Plot of retention time *vs.* EtOH mole fraction: toluene (■) and phenol (○). Five PAA membrane cartridges were used as the chromatographic column. Flow rate, injected sample volume and detection wavelength were the same as in Fig. 2.

The nonlinearity of the curve in Fig. 4 is due to preferential solvation.[24,25] When a polar solute is dissolved in a binary solvent mixture, it interacts differently with each of the solvent components. As a result of the difference in solute–solvent interaction, the solvent composition in the near vicinity of the solute is different from the bulk solvent composition. In an EtOH–heptane binary mixture, the solvent composition of a solute molecule's solvation shell is enriched in EtOH molecules. In Fig. 4, the change of absorption maximum of phenol is similar to the change in retention time. Thus, the preferential solvation of phenol is one of the causes of the sharp retention time change in the low EtOH mole fraction. With increasing EtOH mole fraction, phenol is solvated by more EtOH molecules, which weakens the interaction between phenol and the alumina surface.

Phenol and other alcohol molecules are known to interact with the alumina surface,[26] and these interactions are competitive.

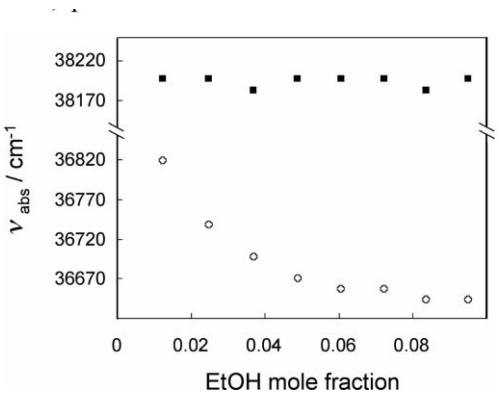

Fig. 4 EtOH mole fraction dependence of absorption maxima in bulk solution: toluene (■) and phenol (○). Solute concentrations were 2×10^{-4} M.

Therefore, when the EtOH mole fraction of the mobile phase increases, the interaction between phenol and the alumina surface is reduced by not only solvation of phenol but also interactions between alumina and EtOH molecules. The decrease in retention time is thought to be a result of both preferential solvation and the interaction between EtOH and alumina. At EtOH mole fractions above 0.04, phenol is sufficiently solvated by EtOH molecules, and many EtOH molecules interact with the alumina surface. As a consequence, the retention time of phenol becomes equal to that of toluene.

In conclusion, PAA membranes can function as a chromatographic column in normal-phase HPLC. The retention of solute is strongly affected by the number of membranes and solvation in the vicinity of the solutes and alumina surface, which indicates that retention can be controlled by changing the number of membranes and the solvent composition of mobile phase. By using PAA as a chromatographic column, it is possible that analysis systems such as analytical chips can be greatly downsized.

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